# Risk factors for bloodstream infections due to colistin-resistant KPC-producing *Klebsiella pneumoniae:* results from a multicenter case–control–control study

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## Abstract

The increasing prevalence of colistin resistance (ColR) *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* (Kp) is a matter of concern because of its unfavourable impact on mortality of KPC-Kp bloodstream infections (BSI) and the shortage of alternative therapeutic options. A matched case–control–control analysis was conducted. The primary study end point was to assess risk factors for ColR KPC-Kp BSI. The secondary end point was to describe mortality and clinical characteristics of these infections. To assess risk factors for ColR, 142 patients with ColR KPC-Kp BSI were compared to two controls groups: 284 controls without infections caused by KPC-Kp (control group A) and 284 controls with colistin-susceptible (ColS) KPC-Kp BSI (control group B). In the first multivariate analysis (cases vs. group A), previous colistin therapy, previous KPC-Kp colonization, $\geq$3 previous hospitalizations, Charlson score $\geq$3 and neutropenia were found to be associated with the development of ColR KPC-Kp BSI. In the second multivariate analysis (cases vs. group B), only previous colistin therapy, previous KPC-Kp colonization and Charlson score $\geq$3 were associated with ColR. Overall, ColR among KPC-Kp blood isolates increased more than threefold during the 4.5-year study period, and 30-day mortality of ColR KPC-Kp BSI was as high as 51%. Strict rules for the use of colistin are mandatory to staunch the dissemination of ColR in KPC-Kp-endemic hospitals.


**Keywords:** Bloodstream infection, colistin resistance, *Klebsiella*, KPC, risk factors
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## Introduction

Bloodstream infections (BSI) due to *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* (KPC-Kp) have become an important problem in many parts of the



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world, including Italy, and are associated with high mortality (40–60%) [1–9].

A favourable effect in terms of reduced mortality has been observed in patients treated with colistin-based combination therapy [1–4]. However, colistin resistance (ColR) among KPC-Kp has been increasingly reported, especially in southern Europe, and an association between reduced susceptibility to colistin and increased mortality has been suggested [10–15]. In this worrisome scenario, ColR might thus further threaten patient survival from infections due to carbapenem-resistant *Enterobacteriaceae* [10–12,16,17].

The aim of this multicenter study was to assess risk factors for BSI due to colistin-resistant (ColR) KPC-Kp, as well as to describe mortality and clinical characteristics of these infections.

## Materials and Methods

### Setting, study design and patients

From January 2010 to June 2014, a retrospective study was conducted in six large, full-service teaching hospitals in Italy. A case–control–control design was used to study risk factors for ColR KPC-Kp BSI. All adult patients (≥18 years old) who developed a monomicrobial ColR KPC-Kp BSI during the study period were defined as cases. A ColR KPC-Kp BSI was defined as the presence of at least one ColR KPC-Kp–positive blood culture with concomitant signs and symptoms of infection. The case was defined as healthcare-associated or hospital-acquired infection according to standard definitions [18,19]. Cases were identified *via* databases maintained by the microbiology laboratories of the six hospitals, together with chart review. Each patient was included in the study only once, at the time of the first KPC-Kp isolation from blood.

To assess risk factors for ColR KPC-Kp BSI, cases were compared to two control populations. The first control group (control group A) included patients without KPC-Kp infections, defined as patients without BSI or KPC-Kp-positive culture of any type during their index hospitalization; the second control group comprised patients with monomicrobial colistin-susceptible (ColS) KPC-Kp BSI (control group B). Two controls per case were included in both populations. Controls were matched to cases by participating hospital, type of ward (medical, surgical or intensive care), date of hospital admission (±1 month) and time at risk (±7 days). In patients with KPC-Kp BSI (cases and group B) time at risk was defined as the number of days elapsing from hospital admission to the date of the first blood culture positive for KPC-Kp, while in patients without KPC-Kp infections (group A) time at risk was defined as the number of days elapsing from hospital admission to hospital discharge or in-hospital death.

In addition to demographic data, other factors analysed as possible risk factors for ColR KPC-Kp BSI included comorbidities (also collectively expressed on the basis of Charlson index) [20], presence of indwelling devices, previous isolation of multidrug-resistant bacteria, previous colonization with KPC-Kp (defined as a KPC-Kp isolation from nonsterile sites during previous hospitalizations in the absence of signs and symptoms of infection) and several aspects of the patient's recent medical history (e.g. contacts with the healthcare system, receipt of immunosuppressive therapies, receipt of chemotherapy/radiotherapy, previous antibiotic therapy). Table 1 lists the variables evaluated as possible risk factors for ColR KPC-Kp BSI. Other variables, evaluated in patients with KPC-Kp BSI only for descriptive purposes, were presentation with septic shock, severity of illness at infection onset as reflected by the Pitt bacteremia score [21], source of infection, adequacy of empirical antimicrobial treatment (empirical treatment was defined as inadequate unless it included at least one drug displaying *in vitro* activity against the KPC-Kp isolate), the number and type of drugs included in the postantibiogram treatment regimen (depending on the number of *in vitro*–active drugs they included (1 or >1), treatment regimens were classified as monotherapy or combination therapy), clinical response to postantibiogram therapy (with treatment failure being defined as death, or persistence or worsening of signs of infection after 72 hours of postantibiogram therapy) and survival at 30 days after the positive blood culture.

### Microbiological procedures

Isolates were identified with the Vitek 2 system (bioMérieux, Marcy l'Etoile, France) and/or by matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry (MALDI Biotyper, Bruker Daltonics, Leipzig, Germany, or Vitek-MS, bioMérieux). The *in vitro* susceptibility of the isolates was assessed with the Vitek 2 system (bioMérieux) or the Sensititre broth microdilution method (Trek Diagnostic Systems, Cleveland, OH, USA). Results were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf). The breakpoints used for defining nonsusceptibility to colistin, ertapenem and meropenem were as follows: minimum inhibitory concentration (MIC) >2 μg/mL, >0.5 μg/mL and >2 μg/mL, respectively. The presence of carbapenemase genes of $bla_{KPC}$, $bla_{NDM}$, $bla_{VIM}$ and $bla_{OXA-48}$ types was determined by PCR reaction and DNA sequencing analysis using previously described protocols [22,23].

### Statistical analysis

The study primary aim was to assess factors associated with ColR KPC-BSI. To this aim, the aforementioned variables were

**TABLE 1. Univariate analysis of risk factors for BSI caused by colistin-resistant KPC-Kp (case group) compared to control patients without infections caused by KPC-Kp (control group A) and control patients with BSI caused by colistin-susceptible KPC-Kp (control group B)**

| Variable | Case group ($n$ = 142) | Control group A ($n$ = 284) | Case group vs. control group A | | Control group B ($n$ = 284) | Case group vs. control group B | |
|---|---|---|---|---|---|---|---|
| | | | OR (95% CI) | p | | OR (95% CI) | p |
| Age (years), median (IQR) | 66 (54–74) | 70 (54–78) | — | 0.06 | 67 (55–77) | — | 0.23 |
| Male sex | 89 (62.7) | 164 (57.7) | 1.23 (0.80–1.90) | 0.33 | 180 (63.4) | 0.97 (0.63–1.51) | 0.89 |
| Comorbidities | | | | | | | |
| COPD | 14 (9.9) | 46 (16.2) | 0.56 (0.28–1.10) | 0.08 | 41 (14.4) | 0.65 (0.31–1.27) | 0.18 |
| Biliary devices | 1 (0.7) | 5 (1.8) | 0.39 (0.01–3.59) | 0.38 | 7 (2.5) | 0.28 (0.01–2.22) | 0.21 |
| Cardiovascular diseases | 53 (37.3) | 130 (45.8) | 0.70 (0.46–1.09) | 0.10 | 120 (42.2) | 0.81 (0.52–1.25) | 0.33 |
| Cerebrovascular diseases and dementia | 15 (10.6) | 43 (15.1) | 0.66 (0.33–1.27) | 0.19 | 35 (12.3) | 0.84 (0.41–1.65) | 0.59 |
| Solid organ cancer | 26 (18.3) | 65 (22.9) | 0.75 (0.43–1.28) | 0.28 | 50 (17.6) | 1.05 (0.59–1.82) | 0.86 |
| Hematologic cancer | 26 (18.3) | 25 (8.8) | 2.32 (1.23–4.38) | 0.004 | 43 (15.1) | 1.26 (0.70–2.21) | 0.40 |
| Diabetes mellitus | 30 (21.1) | 54 (19.0) | 1.14 (0.66–1.93) | 0.60 | 71 (25.0) | 0.80 (0.48–1.33) | 0.37 |
| Splenectomy | 2 (1.4) | 3 (1.1) | 1.34 (0.11–11.81) | 0.75 | 4 (1.4) | 1 (0.09–7.07) | 1.00 |
| Chronic renal failure | 23 (16.2) | 35 (12.3) | 1.37 (0.74–2.51) | 0.27 | 54 (19.0) | 0.82 (0.46–1.44) | 0.48 |
| Liver disease | 13 (9.1) | 14 (4.9) | 1.94 (0.81–4.59) | 0.09 | 27 (9.5) | 0.96 (0.44–2.00) | 0.91 |
| HIV infection | 3 (2.1) | 3 (1.1) | 2.02 (0.27–15.26) | 0.38 | 13 (4.6) | 0.45 (0.08–1.68) | 0.21 |
| Solid organ transplantation | 13 (9.1) | 5 (1.8) | 5.62 (1.82–20.49) | <0.001 | 21 (7.4) | 1.26 (0.56–2.74) | 0.53 |
| Charlson score ≥3 | 41 (28.9) | 36 (12.7) | 2.79 (1.63–4.77) | <0.001 | 39 (13.7) | 2.25 (1.50–4.31) | <0.001 |
| History | | | | | | | |
| Previous hospitalization | 94 (66.2) | 97 (34.1) | 3.77 (2.41–5.92) | <0.001 | . | 1.24 (0.80–1.93) | 0.32 |
| ≥1 | 79 (55.6) | 98 (34.5) | 2.38 (1.54–3.67) | <0.001 | 121 (42.6) | 1.69 (1.10–2.59) | 0.01 |
| ≥2 | 48 (33.8) | 36 (12.7) | 3.52 (2.08–5.94) | <0.001 | 60 (21.1) | 1.91 (1.18–3.06) | 0.005 |
| ≥3 | 30 (21.1) | 15 (5.3) | 4.80 (2.39–9.96) | <0.001 | 36 (12.7) | 1.84 (1.04–3.25) | 0.02 |
| ≥4 | 15 (10.6) | 11 (3.9) | 2.93 (1.21–7.25) | 0.006 | 26 (9.1) | 1.17 (0.56–2.39) | 0.64 |
| ≥5 | 12 (8.4) | 10 (3.5) | 2.53 (0.97–6.70) | 0.03 | 19 (6.7) | 1.29 (0.55–2.89) | 0.51 |
| Outpatient follow-up | 32 (22.5) | 70 (24.6) | 0.89 (0.53–1.46) | 0.63 | 49 (17.2) | 1.39 (0.81–2.36) | 0.19 |
| Admission from another healthcare facility | 3 (2.1) | 4 (1.4) | 1.51 (0.22–9.05) | 0.59 | 19 (6.7) | 0.30 (0.06–1.05) | 0.04 |
| Previous ICU admission | 38 (26.8) | 12 (4.2) | 8.28 (4.02–18.01) | <0.001 | 54 (19.0) | 1.56 (0.94–2.57) | 0.07 |
| Intravenous home therapy | 2 (1.4) | 2 (0.7) | 2.01 (0.14–28.00) | 0.48 | 2 (0.7) | 2.01 (0.14–28.00) | 0.48 |
| Previous urinary tract infection | 19 (13.4) | 20 (7.0) | 2.04 (0.99–4.17) | 0.03 | 42 (14.8) | 0.89 (0.47–1.64) | 0.69 |
| Recent bacterial infections | 67 (47.2) | 47 (16.5) | 4.50 (2.79–7.28) | <0.001 | 93 (32.7) | 1.83 (1.19–2.83) | 0.004 |
| Previous MRSA isolation | 5 (3.5) | 5 (1.8) | 2.04 (0.46–8.99) | 0.26 | 13 (4.6) | 0.76 (0.21–2.33) | 0.61 |
| Previous ESBL-producer isolation | 12 (8.4) | 4 (1.4) | 6.46 (1.90–27.88) | <0.001 | 20 (7.0) | 1.22 (0.52–2.71) | 0.60 |
| Previous VRE isolation | 1 (0.7) | 2 (0.7) | 1.00 (0.02–19.36) | 1.00 | 3 (1.1) | 0.66 (0.01–8.36) | 0.72 |
| Previous KPC-Kp colonization | 51 (35.9) | 8 (2.8) | 19.39 (8.62–48.57) | <0.001 | 49 (17.2) | 2.69 (1.64–4.37) | <0.001 |
| Neutropenia | 16 (11.3) | 12 (4.2) | 2.88 (1.23–6.86) | 0.006 | 37 (13.0) | 0.85 (0.42–1.63) | 0.60 |
| CVC | 97 (68.3) | 94 (33.1) | 4.36 (2.77–6.88) | <0.001 | 175 (61.6) | 1.34 (0.86–2.11) | 0.17 |
| Nasogastric tube | 42 (29.6) | 45 (15.8) | 2.23 (1.34–3.71) | <0.001 | 61 (21.5) | 1.53 (0.94–2.48) | 0.06 |
| Surgical drainage | 21 (14.8) | 28 (9.9) | 1.59 (0.82–3.03) | 0.13 | 51 (18.0) | 0.79 (0.43–1.41) | 0.41 |
| Urinary catheter | 85 (59.9) | 125 (44.0) | 1.90 (1.23–2.92) | 0.002 | 159 (56.0) | 1.17 (0.76–1.80) | 0.45 |
| Endoscopy | 14 (9.9) | 40 (14.1) | 0.67 (0.32–1.31) | 0.22 | 30 (10.6) | 0.93 (0.44–1.88) | 0.82 |
| Mechanical ventilation | 48 (33.8) | 101 (35.6) | 0.92 (0.59–1.45) | 0.72 | 98 (34.5) | 0.97 (0.62–1.51) | 0.88 |
| Dialysis | 21 (14.8) | 5 (1.8) | 9.68 (3.43–33.45) | <0.001 | 41 (14.4) | 1.03 (0.55–1.87) | 0.92 |
| Total parenteral nutrition | 31 (21.8) | 41 (14.4) | 1.65 (0.95–2.86) | 0.05 | 83 (29.2) | 0.68 (0.41–1.11) | 0.10 |
| Immunosuppressive therapy | 18 (12.7) | 12 (4.2) | 3.29 (1.44–7.71) | 0.001 | 25 (8.8) | 1.50 (0.74–2.99) | 0.21 |
| Corticosteroid therapy | 22 (15.5) | 42 (14.8) | 1.06 (0.57–1.91) | 0.85 | 46 (16.2) | 0.95 (0.52–1.70) | 0.85 |
| Chemotherapy/radiotherapy | 18 (12.7) | 69 (24.3) | 0.45 (0.24–0.81) | 0.005 | 39 (13.7) | 0.91 (0.47–1.71) | 0.76 |
| PEG | 4 (2.8) | 5 (1.8) | 1.62 (0.31–7.63) | 0.47 | 8 (2.8) | 1.00 (0.22–3.81) | 1.00 |
| Bedridden | 30 (21.1) | 64 (22.5) | 0.92 (0.54–1.54) | 0.74 | 51 (18.0) | 1.22 (0.71–2.08) | 0.43 |
| Previous surgery | 63 (44.4) | 72 (25.3) | 2.35 (1.50–3.67) | <0.001 | 120 (42.2) | 1.09 (0.71–1.67) | 0.68 |
| Recent antibiotic therapy | | | | | | | |
| In general | 119 (83.8) | 210 (73.9) | 1.82 (1.06–3.21) | 0.02 | 240 (84.5) | 0.95 (0.53–1.73) | 0.85 |
| By classes | | | | | | | |
| Aminoglycosides | 15 (10.6) | 13 (4.6) | 2.46 (1.05–5.79) | 0.02 | 30 (10.6) | 1.00 (0.48–2.00) | 1.00 |
| β-Lactam-β-lactamase inhibitor | 53 (37.3) | 109 (38.4) | 0.96 (0.62–1.48) | 0.83 | 150 (52.8) | 0.53 (0.34–0.82) | 0.002 |
| Fluoroquinolones | 47 (33.1) | 87 (30.6) | 1.12 (0.71–1.76) | 0.60 | 95 (33.4) | 0.98 (0.62–1.54) | 0.94 |
| Oxyiminocephalosporins | 19 (13.4) | 70 (24.6) | 0.47 (0.26–0.84) | 0.007 | 38 (13.4) | 1.00 (0.52–1.87) | 1.00 |
| Carbapenems | 45 (31.7) | 33 (11.6) | 3.53 (2.06–6.05) | <0.001 | 92 (32.4) | 0.97 (0.61–1.52) | 0.88 |
| Glycopeptides | 39 (27.5) | 37 (13.0) | 2.53 (1.47–4.32) | <0.001 | 62 (21.8) | 1.35 (0.83–2.21) | 0.20 |
| Colistin | 40 (28.2) | 6 (2.1) | 18.17 (7.31–53.62) | <0.001 | 15 (5.3) | 7.03 (3.60–14.25) | <0.001 |
| Other | 49 (34.5) | 61 (21.5) | 1.93 (1.20–3.08) | 0.004 | 80 (28.2) | 1.34 (0.85–2.11) | 0.18 |

Data are expressed as $n$ (%) unless stated otherwise.
BSI, bloodstream infection; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CVC, central venous catheter; ESBL, extended-spectrum β-lactamase; HIV, human immunodeficiency virus; ICU, intensive care unit; KPC-Kp, KPC-producing *Klebsiella pneumoniae;* IQR, interquartile range; MRSA, methicillin-resistant *Staphylococcus aureus*; OR, odds ratio; PEG, percutaneous endoscopic gastrostomy; VRE, vancomycin-resistant enterococci.

compared by the Mann-Whitney $U$ test, the Student's $t$ test, the $\chi^2$ test or the Fisher's exact test, as appropriate. These tests were performed for both comparisons (cases vs. control group A and cases vs. control group B), and they were all two sided. Once a univariate statistic was generated, variables with a p value of <0.20 were included in two backward stepwise logistic regression models (cases vs. control group A and cases vs. control group B). The discrimination ability of the models was assessed by estimating the area under the receiver operating characteristic (ROC) curve. Calibration of the models was assessed using the Hosmer-Lemeshow test for goodness of fit. Finally, to describe the outcome throughout

the 30th day after the first positive blood culture, survival distribution functions of both ColR and ColS KPC-Kp BSI were estimated using the Kaplan-Meier product-limit method, then compared using nonparametric log-rank and Wilcoxon tests. Outcomes and clinical features in control group A patients were not reported. All statistical analyses were performed by Intercooled Stata 11 for Windows (StataCorp, College Station, TX, USA).

## Results

During the study period, 729 BSIs due to KPC-Kp were observed, of which 142 (19.5%) and 587 (80.5%) were due to ColR and ColS strains, respectively. As shown in Fig. 1, the rate of ColR among all KPC-Kp BSI increased significantly over time, and this was true for all participating hospitals ($\chi^2$ for trend, $p < 0.001$). In terms of *in vitro* resistance, all ColR isolates were resistant to penicillins, cephalosporins, ertapenem, ciprofloxacin, amikacin, cotrimoxazole and chloramphenicol. Meropenem MICs were ≥16 mg/L for more than two-thirds of ColR KPC-Kp strains (94/142, 66.2%). As many as 116/142 (81.7%) and 111/142 (78.2%) ColR isolates were still susceptible to gentamicin and tigecycline, respectively. However, from 2010 to the first 6 months of 2014, KPC-Kp resistance to gentamicin and tigecycline increased from 6.3% to 20% (p 0.01) and from 9.4% to 25.3% (p 0.01), respectively.

To understand the factors associated with the development of ColR KPC-Kp BSI, cases were compared both with 284 patients without KPC-Kp infections (group A) and with 284 patients with ColS KPC-Kp BSI (group B). Controls were matched with cases as defined above; however, with regard to time at risk, two controls (group B) had time at risk longer (i.e. >7 days) and five controls (group B) shorter (i.e. <7 days) than their case counterpart.

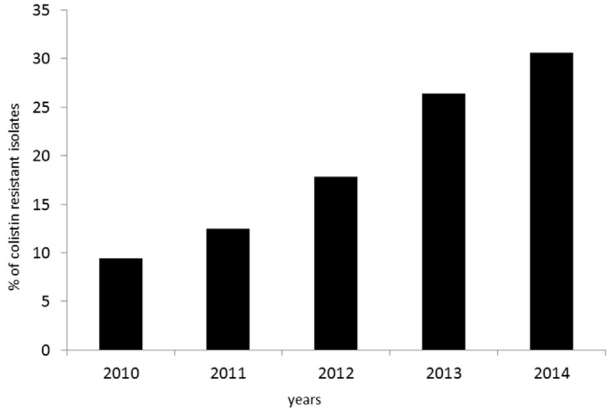


**FIG. 1.** Increase in colistin resistance (ColR) among blood *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* isolates during the study period ($\chi^2$ for trend, $p < 0.001$).

### Univariate analysis

As shown in Table 1, in the univariate analysis comparing cases versus group A controls without KPC-Kp infection, significant factors included the following: receipt of a solid organ transplant, Charlson score, number of previous hospitalizations, previous intensive care unit (ICU) admission, previous bacterial infections (excluding KPC-Kp), previous KPC-Kp colonization, neutropenia, presence of indwelling devices, dialysis, total parenteral nutrition, immunosuppressive therapy, chemo- and radiotherapy, previous surgery and previous antibiotic therapy with aminoglycosides, cephalosporins, carbapenems, glycopeptides and colistin. When cases were compared to ColS KPC-Kp BSI (control group B), only Charlson score, number of previous hospitalizations, previous bacterial infections, previous KPC-Kp colonization and use of colistin were found to be significantly different between the two groups. Transfer from another healthcare facility, which was not significant in the cases versus control A analysis, had a significant p value in the cases versus control B analysis.

### Multivariate analyses

As shown in Table 2, in the multivariate comparison of cases vs. control group A (no KPC-Kp infection), factors that remained in the final model were previous colistin therapy (odds ratio (OR), 24.51, p <0.001), previous KPC-Kp colonization (OR, 18.71, p <0.001), ≥3 previous hospitalizations (OR, 5.32, p <0.001), Charlson score ≥3 (OR, 2.84, p 0.001) and neutropenia (OR, 2.72, p 0.04). Table 2 also shows the multivariate comparison of cases versus control group B (ColS KPC-Kp BSI). In this analysis, previous colistin administration was confirmed as a predictor of ColR KPC-Kp BSI (OR, 6.88, p <0.001), along with previous KPC-Kp colonization (2.40, p 0.001) and Charlson score ≥3 (OR, 2.97, p <0.001). Both multivariate models displayed good predictive ability because the ROC area under the curve was 0.80 for the first model (cases vs. control group A) and 0.75 for the second (cases vs. control group B). The results of Hosmer-Lemshow chi-square testing ($\chi^2$ = 22.8; p 0.24 for the first model and $\chi^2$ = 1.15; p 0.88 for the second model) were indicative of good calibration.

### Clinical features of ColR and ColS KPC-Kp BSI and outcome

As shown in Table 3, ColR KPC-Kp BSI were more often associated with the lower respiratory tract as the source of infection and inadequate empirical antibiotic treatment than ColS KPC-Kp BSI. In general, ColR received a lower percentage

**TABLE 2. Multivariate analysis of risk factors for BSI caused by colistin-resistant KPC-Kp**[a]

| Control group and risk factors | OR (95% CI) | p |
|---|---|---|
| Control group A (patients without KPC-Kp infection)[b] | | |
| Previous colistin administration | 24.51 (8.75–68.67) | <0.001 |
| Previous colonization with KPC-Kp | 18.71 (8.05–43.51) | <0.001 |
| Previous ≥3 hospitalization | 5.32 (2.48–11.38) | <0.001 |
| Charlson score ≥3 | 2.84 (1.52–5.29) | 0.001 |
| Neutropenia | 2.72 (1.02–7.23) | 0.04 |
| Control group B (patients with BSI due to colistin-susceptible KPC-Kp)[c] | | |
| Previous colistin administration | 6.88 (3.55–13.34) | <0.001 |
| Previous colonization with KPC-Kp | 2.40 (1.46–3.97) | 0.001 |
| Charlson score ≥3 | 2.97 (1.74–5.06) | <0.001 |

BSI, bloodstream infection; CI, confidence interval; KPC-Kp, KPC-producing *Klebsiella pneumoniae*; OR, odds ratio.
[a]Only variables retained in the final multivariate models are presented.
[b]Variables with p < 0.20 in the univariate analysis (i.e. age, chronic obstructive pulmonary disease, cardiovascular diseases, cerebrovascular diseases and dementia, hematologic cancer, liver disease, solid organ transplantation, Charlson score ≥3, previous hospitalization, previous ≥1 hospitalization, previous ≥2 hospitalization, previous ≥3 hospitalization, previous ≥4 hospitalization, previous ≥5 hospitalization, previous intensive care unit admission, previous urinary tract infections, previous extended-spectrum β-lactamases-producer isolation, recent bacterial infections, previous colonization with KPC-Kp, neutropenia, presence of central venous catheter, presence of nasogastric tube, surgical drainage, presence of urinary catheter, dialysis, chemotherapy/radiotherapy, total parenteral nutrition, previous surgery, recent antibiotic therapy, recent therapy with aminoglycosides, recent therapy with oxyiminocephalosporins, recent therapy with carbapenems, recent therapy with glycopeptides, previous colistin administration, recent therapy with antibiotics other than aminoglycosides, β-lactam-β-lactamase inhibitor, fluoroquinolones, colistin, oxyiminocephalosporins and glycopeptides) were considered for the multivariate model of cases vs. control group A.
[c]Variables with p < 0.20 in the univariate analysis (i.e. chronic obstructive pulmonary disease, Charlson score ≥3, previous ≥1 hospitalization, previous ≥3 hospitalization, admission from another healthcare facility, previous intensive care unit admission, recent bacterial infections, previous colonization with KPC-Kp, presence of central venous catheter, presence of nasogastric tube, total parenteral nutrition, recent therapy with β-lactam-β-lactamase inhibitor, previous colistin administration, recent therapy with antibiotics other than aminoglycosides, β-lactam-β-lactamase inhibitor, fluoroquinolones, colistin, oxyiminocephalosporins and glycopeptides) were considered for the multivariate model of cases vs. control group B.

**TABLE 3. Characteristics of 426 patients with BSI caused by KPC-Kp according to colistin resistance (ColR) or colistin susceptibility (ColS) of the isolates**

| Variable | ColR patients, case group (*n* = 142) | ColS patients, control group B (*n* = 284) | p |
|---|---|---|---|
| Type of infection | | | |
| HCA infection | 13 (9.1) | 27 (9.5) | 0.96 |
| Hospital-acquired infection | 129 (90.8) | 257 (90.5) | 0.91 |
| Septic shock | 29 (20.4) | 53 (18.7) | 0.66 |
| Pitt bacteremia score ≥4 | 53 (37.3) | 82 (28.8) | 0.07 |
| Source of infection | | | |
| Urinary tract | 25 (17.6) | 69 (24.3) | 0.12 |
| Lower Respiratory tract | 29 (20.4) | 34 (11.9) | 0.02 |
| Pancreas and biliary tract | 3 (2.1) | 8 (2.8) | 0.66 |
| Central venous catheter | 36 (25.3) | 62 (21.8) | 0.41 |
| Surgical wound | 13 (9.1) | 28 (9.9) | 0.82 |
| Other | 5 (3.5) | 11 (3.9) | 0.86 |
| Unknown | 40 (28.2) | 89 (31.3) | 0.50 |
| Inadequate empirical antimicrobial treatment | 94 (66.2) | 155 (54.6) | 0.02 |
| Postantibiogram therapy | | | |
| Colistin-including therapy | 14 (9.8) | 208 (73.2) | <0.001 |
| Tigecycline-including therapy | 79 (55.6) | 145 (51.1) | 0.37 |
| Gentamicin-including therapy | 66 (46.5) | 77 (27.1) | <0.001 |
| Monotherapy | 47 (33.1) | 53 (18.6) | <0.001 |
| Combination therapy | 95 (66.9) | 231 (81.3) | <0.001 |
| Two-drug combinations | 36 (25.3) | 78 (27.5) | 0.64 |
| Three-drug combinations | 49 (34.5) | 151 (53.2) | <0.001 |
| Four drug combinations | 10 (7.1) | 2 (0.7) | <0.001 |
| Carbapenem-excluding combinations | 28 (19.7) | 72 (25.4) | 0.19 |
| Carbapenem-including combinations | 67 (47.2) | 159 (55.9) | 0.08 |
| Double-carbapenem combinations | 15 (10.6) | 6 (2.1) | <0.001 |
| Rifampin addition to combinations | 11 (7.7) | 2 (0.7) | <0.001 |
| Outcome parameters | | | |
| Initial treatment failure | 38 (26.7) | 42 (14.8) | 0.003 |
| Death | 73 (51.4) | 112 (39.4) | 0.02 |
| Time to discharge (days), median (IQR)[a] | 27 (18–40) | 20 (14–37) | 0.04 |

Data are expressed as *n* (%) unless stated otherwise.
BSI, bloodstream infections; HCA, healthcare associated; KPC-Kp, KPC-producing *Klebsiella pneumoniae*; IQR, interquartile range.
[a]Time to discharge was defined as time (days) from date of index blood culture to discharge of patients and calculated on patients who survived only.

of combination therapies, and in particular a lower number of three-drug combinations. Gentamicin, double carbapenem combinations and rifampin were more frequently used in ColR KPC-Kp. ColR KPC-Kp BSI appeared to be associated with a higher percentage of initial treatment failure (assessed at 72 hours after beginning of therapy) and of mortality than ColS KPC-Kp BSI. Indeed, the overall 30-day crude mortality was 51% (73/142) versus 39.4% (112/284) for ColR and ColS cases, respectively (p 0.02). The difference in mortality was also shown by the Kaplan-Meier survival estimates, although the two curves start to divaricate only after the eighth day after the first positive blood culture (Fig. 2).

## Discussion

The increasing prevalence of ColR among KPC-Kp is of great concern because of its apparent unfavourable impact on mortality in KPC-Kp BSI and the dramatic shortage of alternative therapeutic options [10,11,14,24]. To our knowledge, this is the largest sample of patients with BSI due to ColR KPC-Kp reported to date. The case–control–control design we applied to this study allowed a reliable comparison between variables associated with ColS and ColR KPC-Kp BSI. The two pivotal observations stemming from this study are, first, that among all KPC-Kp BSI, ColR increased more than threefold during the 4.5-year study period, and that previous colistin administration was the strongest factor differentiating ColR cases from both control groups.

The association between colistin administration and ColR was already observed in three previous single-center studies. Matthaiou *et al.* [16] reported 41 patients colonized or infected with colistin-resistant Gram-negative rods, of which 33 were

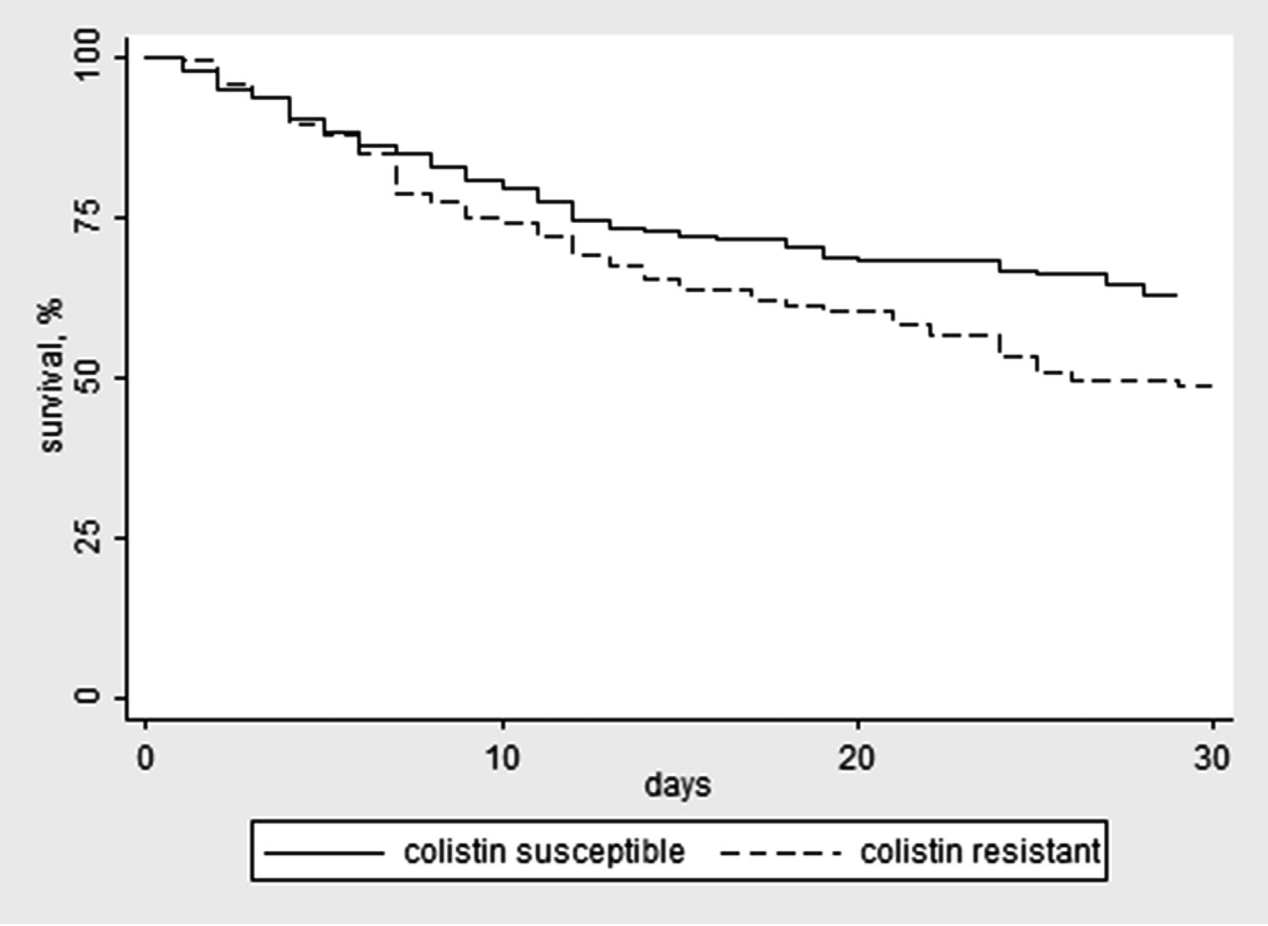


**FIG. 2.** Kaplan-Meier survival curves of patients with bloodstream infection due to *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* according to colistin resistance or susceptibility of isolates.

*K. pneumoniae*. Although various conditions, including age and length of stay in the ICU, were initially associated with resistance, only previous colistin treatment remained in the final multivariate model. Similarly, Kontopidou *et al.* [17] found an association between previous colistin therapy and subsequent colonization with ColR KPC-Kp. Finally, previous colistin therapy was an independent predictor of ColR KPC-Kp isolation among 254 ICU patients in another Greek study [25]. Therefore, although probably slightly overestimated in the comparison between cases and control group B [26], the role of previous colistin therapy as an independent risk factor for ColR KPC-Kp BSI was strongly confirmed in both multivariate models of our study, underscoring the absolute need of avoiding unnecessary colistin use in clinical practice. This requires the application of strict rules for the initiation and, perhaps more importantly, early discontinuation of colistin in clinical practice in hospitals that are endemic for KPC-Kp infections.

Previous colistin administration is not the only factor involved in the development of ColR, since 72% of BSI caused by ColR KPC-Kp developed in patients without a history of colistin administration. Indeed, in both multivariate models (cases vs. control group A and cases vs. control group B), the occurrence of ColR KPC-Kp BSI was also associated with previous colonization with KPC-Kp. Although we cannot exclude the notion that the two variables are somewhat interconnected (because some colonized patients might have received colistin), there is the possibility that these patients might have been primarily colonized with ColR strains. Cross-transmission of both ColS and ColR strains, irrespectively of any previous antibiotic administration, has been demonstrated [25]. In line with this hypothesis, in our study, the rate of previous hospitalizations (and consequently the risk of colonization) did not significantly differ between ColS and ColR patients, but it was higher in ColR patients compared to controls without KPC-Kp infections.

It is of note that ColR patients had increased Charlson scores compared to both ColS ($p < 0.001$) and patients without KPC-Kp infections (p 0.001). The underlying diseases and their

 

severity are often a silent but disturbing presence in this setting and always play a pivotal role in the development of bacterial and fungal severe infections. The great improvements of modern medicine and surgery have led to increased patient survival from many severe diseases; however, this requires intensive therapies, immunosuppression and repeated hospitalizations, thus increasing the risk of acquisition of resistant organisms and the administration of repeated, more or less appropriated antibiotic therapies.

Consistently with previous reports [10,11], in our study the mortality rate appeared to be higher in ColR than ColS KPC-Kp BSI (51% vs. 39%). This finding supports the independent role of ColR as a predictor of mortality, which has been already observed in our cohort [10]. In this regard, the development of ColR further reduces the already poor therapeutic armamentarium for these patients, because colistin, more than other drugs (tigecycline and gentamicin), is the backbone of therapy for KPC-Kp infections, and this might have adversely affected survival in our cohort. However, further studies with appropriate methodology are needed to accurately explore risk factors for mortality of ColR KPC-Kp BSI and to adequately weigh the role of antimicrobial therapy against this backdrop.

This study has other limitations. Among them, we should mention the fact that other unmeasured factors such as previous colistin exact duration and type of treatment (monotherapy vs. combination therapy) might have significantly contributed to the emergence of ColR strains. We could not control for the role of cross-transmission from colonized patients in adjacent beds, a factor that has been previously shown to be associated with ColR KPC-Kp acquisition [25]. However, cases and controls were matched for hospital, ward, month of admission and time at risk. Therefore, the relevance of cross-transmission during the study period is unlikely to have been strongly underestimated [27]. Moreover, we indirectly evaluated the impact of cross-transmission during previous hospitalizations through the case–control–control design of the study, as previously explained.

In conclusion, the use of colistin other than for the treatment of culture-proven or highly suspected ColS KPC-Kp invasive infections should be avoided. Obviously, there is an urgent need for new antibiotics for treating these desperately ill patients, but in the meantime, there is an even more urgent need to staunch the dissemination of these resistant organisms. Reports like this one might be pivotal for understanding the phenomenon of increased antibiotic resistance, but they also testify to the failure of our not negligible efforts to highlight the dissemination of antibiotic resistance, which is endangering the incredible improvements of modern medicine and surgery. To counteract this trend, active, centrally driven and funded programs are urgently needed.

## Transparency Declaration

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## References

[1] Neuner EA, Yeh JY, Hall GS, et al. Treatment and outcomes in carbapenem-resistant *Klebsiella pneumoniae* bloodstream infections. Diagn Microbiol Infect Dis 2011;69:357–62.

[2] Pontikis K, Karaiskos I, Bastani S, et al. Outcomes of critically ill intensive care unit patients treated with fosfomycin for infections due to pandrug-resistant and extensively drug-resistant carbapenemase-producing Gram-negative bacteria. Int J Antimicrob Agents 2014;43:52–9.

[3] Qureshi ZA, Paterson DL, Potoski BA, et al. Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae*: superiority of combination antimicrobial regimens. Antimicrob Agents Chemother 2012;56:2108–13.

[4] Tumbarello M, Viale P, Viscoli C, et al. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: importance of combination therapy. Clin Infect Dis 2012;55:943–50.

[5] Giannella M, Trecarichi EM, De Rosa FG, et al. Risk factors for carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection among rectal carriers: a prospective observational multicentre study. Clin Microbiol Infect 2014;20:1357–62.

[6] Daikos GL, Tsaousi S, Tzouvelekis LS, et al. Carbapenemase-producing *Klebsiella pneumoniae* bloodstream infections: lowering mortality by antibiotic combination schemes and the role of carbapenems. Antimicrob Agents Chemother 2014;58:2322–8.

[7] Giacobbe DR, Del Bono V, Marchese A, et al. Early carbapenem-resistant *Klebsiella pneumoniae* bacteraemia: should we expand the screening? Clin Microbiol Infect 2014;20:O1157–8.

[8] Bogdanovich T, Adams-Haduch JM, Tian GB, et al. Colistin-resistant, *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* belonging to the international epidemic clone ST258. Clin Infect Dis 2011;53:373–6.

[9] Marchaim D, Chopra T, Pogue JM, et al. Outbreak of colistin-resistant, carbapenem-resistant *Klebsiella pneumoniae* in metropolitan Detroit, Michigan. Antimicrob Agents Chemother 2011;55:593–9.

[10] Tumbarello M, Trecarichi EM, De Rosa FG, et al. Infections caused by KPC-producing *Klebsiella pneumoniae*: differences in therapy and mortality in a multicentric study. J Antimicrob Chemother 2015;70:2133–43.

[11] Capone A, Giannella M, Fortini D, et al. High rate of colistin resistance among patients with carbapenem-resistant *Klebsiella pneumoniae* infection accounts for an excess of mortality. Clin Microbiol Infect 2013;19:E23–30.

[12] Neonakis IK, Samonis G, Messaritakis H, et al. Resistance status and evolution trends of *Klebsiella pneumoniae* isolates in a university hospital in Greece: ineffectiveness of carbapenems and increasing resistance to colistin. Chemotherapy 2010;56:448–52.

[13] Zarkotou O, Pournaras S, Voulgari E, et al. Risk factors and outcomes associated with acquisition of colistin-resistant KPC-producing *Klebsiella pneumoniae*: a matched case–control study. J Clin Microbiol 2010;48:2271–4.

[14] Monaco M, Giani T, Raffone M, et al., Network EuSCAPE–Italy. Colistin resistance superimposed to endemic carbapenem-resistant

*Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. Euro Surveill 2014;19:pii20939.

[15] Antoniadou A, Kontopidou F, Poulakou G, et al. Colistin-resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: first report of a multiclonal cluster. J Antimicrob Chemother 2007;59:786–90.

[16] Matthaiou DK, Michalopoulos A, Rafailidis PI, et al. Risk factors associated with the isolation of colistin-resistant Gram-negative bacteria: a matched case–control study. Crit Care Med 2008;36:807–11.

[17] Kontopidou F, Plachouras D, Papadomichelakis E, et al. Colonization and infection by colistin-resistant Gram-negative bacteria in a cohort of critically ill patients. Clin Microbiol Infect 2011;17:E9–11.

[18] Friedman ND, Kaye KS, Stout JE, et al. Health care–associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med 2002;137:791–7.

[19] Garner JS, Jarvis WR, Emori TG, et al. CDC definitions for nosocomial infections. Am J Infect Control 1988;16:128–40.

[20] Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373–83.

[21] Rhee JY, Kwon KT, Ki HK, et al. Scoring systems for prediction of mortality in patients with intensive care unit–acquired sepsis: a comparison of the Pitt bacteremia score and the Acute Physiology and Chronic Health Evaluation II scoring systems. Shock 2009;31:146–50.

[22] Endimiani A, Hujer AM, Perez F, et al. Characterization of blaKPC-containing *Klebsiella pneumoniae* isolates detected in different institutions in the eastern USA. J Antimicrob Chemother 2009;63:427–37.

[23] Giani T, Pini B, Arena F, et al., AMCLI-CRE Survey Participants. Epidemic diffusion of KPC carbapenemase-producing *Klebsiella pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011. Euro Surveill 2013;18:pii20489.

[24] Kontopidou F, Giamarellou H, Katerelos P, et al. Infections caused by carbapenem-resistant *Klebsiella pneumoniae* among patients in intensive care units in Greece: a multi-centre study on clinical outcome and therapeutic options. Clin Microbiol Infect 2014;20:O117–1123.

[25] Papadimitriou-Olivgeris M, Christofidou M, Fligou F, et al. The role of colonization pressure in the dissemination of colistin or tigecycline resistant KPC-producing *Klebsiella pneumoniae* in critically ill patients. Infection 2014;42:883–90.

[26] Harris AD, Karchmer TB, Carmeli Y, et al. Methodological principles of case–control studies that analysed risk factors for antibiotic resistance: a systematic review. Clin Infect Dis 2001;2:1055–61.

[27] Tumbarello M, Trecarichi EM, Tumietto F, et al. Predictive models for identification of hospitalized patients harboring KPC-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2014;58:3514–20.